DT04 Rec'd PCT/PTO 15 SEP 2004

Title

A BUILDING BLOCK CAPABLE OF FUNCTIONAL ENTITY TRANSFER TO NUCLEOPHIL

Technical Field of the Invention

The present invention relates to a building block comprising a complementing element and precursor for a functional entity. The building block is designed to transfer the functional entity with an adjustable efficiency to a recipient reactive group upon recognition between the complementing element and an encoding element associated with the reactive group. The invention also relates to a linkage between the functional entity and the complementing element as well as a method for transferring a functional entity to recipient reactive group.

Background

The transfer of a chemical entity from one mono-, di- or oligonucleotide to another has been considered in the prior art. Thus, N. M. Chung *et al.* (Biochim. Biophys. Acta,1971, 228,536-543) used a poly(U) template to catalyse the transfer of an acetyl group from 3'-O-acetyladenosine to the 5'-OH of adenosine. The reverse transfer, i.e. the transfer of the acetyl group from a 5'-O-acetyladenosine to a 3'-OH group of another adenosine, was also demonstrated.

Walder *et al.* Proc. Natl. Acad. Sci. USA, 1979, 76, 51-55 suggest a synthetic procedure for peptide synthesis. The synthesis involves the transfer of nascent immobilized polypeptide attached to an oligonucleotide strand to a precursor amino acid attached to an oligonucleotide. The transfer comprises the chemical attack of the amino group of the amino acid precursor on the substitution labile peptidyl ester, which in turn results in an acyl transfer. It is suggested to attach the amino acid precursor to the 5' end of an oligonucleotide with a thiol ester linkage.

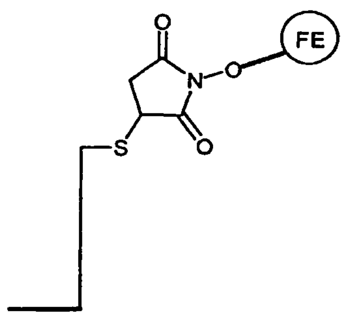
The transfer of a peptide from one oligonucleotide to another using a template is disclosed in Bruick RK et al. Chemistry & Biology, 1996, 3:49-56. The carboxy terminal of the peptide is initially converted to a thioester group and subsequently transformed to an activated thioester upon incubation with Ellman's reagent. The activated thioester is reacted with a first oligo, which is 5'-thiol-terminated, resulting in the formation of a thio-ester linked intermediate. The first oligonucleotide and a

second oligonucleotide having a 3' amino group is aligned on a template such that the thioester group and the amino group are positioned in close proximity and a reaction is effected resulting in a coupling of the peptide to the second oligonucleotide through an amide bond.

The prior art building blocks and methods for transfer have a relatively poor transfer efficiency. Therefore, in an aspect of the present invention an oligonucleotide conjugated to a transferable chemical moiety via a linker is provided, which has an increased ability to transfer a functional entity.

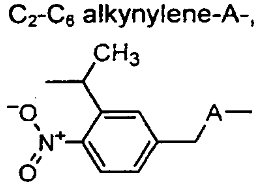
Summary of the Invention

The present invention relates to a building block of the general formula

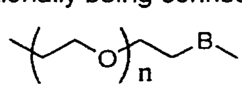


capable of transferring a functional entity (FE) to a recipient reactive group, wherein the lower horizontal line is a **Complementing Element** identifying the functional entity and the vertical line between the complementing element and the S atom is a **Spacer.**

Preferably the spacer is a valence bond, C_1-C_8 alkylene-A-, C_1-C_8 alkenylene-A-, C_2-C_8 alkynylene-A-, or



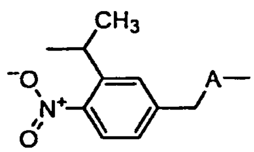
said spacer optionally being connected through A to a moiety selected from

$—(CH_2)_n—B—$, , and

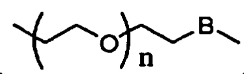
$—(CH_2)_n–S–S–(CH_2)_m–B—$

where A is a valence bond, –C(O)NR^1-, -NR^1-, -O-, -S-, or -C(O)-O-; B is a valence bond, -O-, -S-, –NR^1- or –C(O)NR^1- and connects to the S atom of the carrier; R^1 is selected independently from H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_1-C_6 alkylene-aryl, or aryl substituted with 0-5 halogen atoms selected from -F, -Cl, -Br and –I; and n and m independently are integers ranging from 1 to 10.

In one aspect of the invention the **Spacer** is C_1-C_6 alkylene-A-, C_1-C_6 alkenylene-A-, C_2-C_6 alkynylene-A-, or



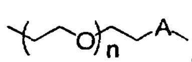
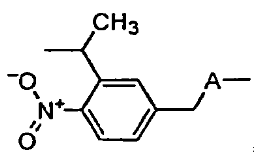
said spacer optionally being connected through A to a moiety selected from

$—(CH_2)_n–B—$,  , and

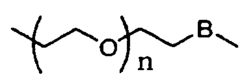
$—(CH_2)_n–S–S–(CH_2)_m–B—$

where A is –C(O)NR^1-, or -S-; B is -S-, –NR^1- or –C(O)NR^1- and connects to S-C-connecting group; R^1 is selected independently from H, C_1-C_6 alkyl, C_1-C_6 alkylene-aryl, or aryl; and n and m independently are integers ranging from 1 to 6.

Preferably the **Spacer** is -A-, a group C_1-C_6 alkylene-A-, C_2-C_6 alkenylene-A-, or C_2-C_6 alkynylene-A- optionally substituted with 1 to 3 hydroxy groups, or

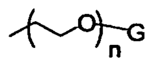
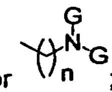
 ,  ,

said spacer being connected through A to a linker selected from

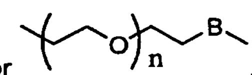
-B-, $—(CH_2)_n–B—$,  , and

$—(CH_2)_n–S–S–(CH_2)_m–B—$

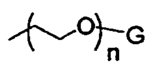
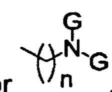
where A is a valence bond, -NR^2-, –C(O)NR^2-, - NR^2-C(O)-, -O-, -S-, -C(O)-O- or –OP(=O)(O$^-$)-O-; B is a valence bond, -O-, -S-, –NR^2-, -C(O)- or –C(O)NR^2- and connects to S-C-connecting group; R^2 is selected independently from H, C_1-C_6 alkyl,

C_3-C_7 cycloalkyl, aryl, C_1-C_6 alkylene-aryl,  or ; G is H or C_1-C_6 alkyl; and n and m independently are integers ranging from 1 to 10.

The spacer may connect to the complementing element in any convenient way. When the complementing element is a nucleic acid, the spacer may connect to the backbone or the nucleobase. In one aspect of the invention, the **spacer** is C_2-C_6 alkenylene-A,
said spacer being connected through A to a moiety selected from

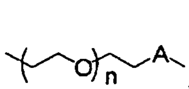
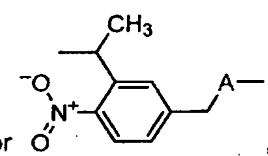
-B-, $—(CH_2)_n–B—$, or ,

where A is a valence bond, –C(O)NR^2-, -NR^2-C(O)-, -S-, -C(O)-O- or –OP(=O)(O$^-$)-O-; B is a valence bond, -S-, –NR^2-, or -C(O)- and connects to S-C-connecting group; n and m independently are integers ranging from 1 to 10 and

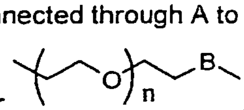
R^2 is selected independently from H,  or , wherein G is H or C_1-C_6 alkyl; and the spacer is connected to the complementing element through a nucleobase.

Suitably, the spacer is attached to the 5 position of a pyrimidine type nucleobase or 7 position of a purine or 7-deaza-purine type nucleobase. However, other position of attachment may be appropriate.

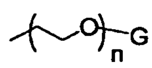
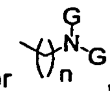
In another aspect of the invention the **spacer** is -A-,

, or ,

said spacer being connected through A to a moiety selected from

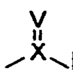
-B-, $—(CH_2)_n–B—$, or ,

where A is a valence bond, -NR^2-C(O)-, -O-, or -S-; B is a valence bond, -S-, -NR^2-, or -C(O)- and connects to S-C-connecting group;
n and m independently are integers ranging from 1 to 10 and

R^2 is selected independently from H,  or , wherein G is H or C_1-C_6 alkyl; and the spacer is connected to the complementing element via a phosphorus group.

The phosphorus group is suitable a phosphate or thiophosphate group attached to a 3' or 5' end of a complementing element.

The building block according to the present invention can transfer a variety of chemical compounds to a recipient reactive group. In one aspect of the invention the functional entity is of the format,  where X = -C-, -S-, -P-, -S(O)-, -P(O)-, and V = O, S, NH, N-C_1-C_6 alkyl. R may be chosen from any chemical group capable of forming a chemical bond to the X atom. In a preferred aspect of the invention

FE is  where

X = -C-, -S-, -P-, -S(O)-, or -P(O)-,

V = O, S, NH, or N-C_1-C_6 alkyl, and

R is H or selected among the group consisting of a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl, and heteroaryl, said group being substituted with 0-3 R^4, 0-3 R^5 and 0-3 R^9 or C_1-C_3 alkylene-NR^4_2, C_1-C_3 alkylene-$NR^4C(O)R^8$, C_1-C_3 alkylene-$NR^4C(O)OR^8$, C_1-C_2 alkylene-O-NR^4_2, C_1-C_2 alkylene-O-$NR^4C(O)R^8$, C_1-C_2 alkylene-O-$NR^4C(O)OR^8$ substituted with 0-3 R^9.

where R^4 is H or selected independently among the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl, heteroaryl, said group being substituted with 0-3 R^9 and

R^5 is selected independently from $-N_3$, -CNO, $-C(NOH)NH_2$, -NHOH, $-NHNHR^6$, $-C(O)R^6$, $-SnR^6_3$, $-B(OR^6)_2$, $-P(O)(OR^6)_2$ or the group consisting of C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl said group being substituted with 0-2 R^7,

where R^6 is selected independently from H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl or C_1-C_6 alkylene-aryl substituted with 0-5 halogen atoms selected from -F, -Cl, -Br, and –I; and R^7 is independently selected from $-NO_2$, $-COOR^6$, $-COR^6$, -CN, $-OSiR^6_3$, $-OR^6$ and $-NR^6_2$.

R^8 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, aryl or C_1-C_6 alkylene-aryl substituted with 0-3 substituents independently selected from –F, -Cl, –NO_2, -R^3, -OR^3, -SiR^3_3

R^9 is =O, -F, -Cl, -Br, -I, -CN, -NO_2, -OR^6, -NR^6_2, -NR^6-C(O)R^8, -NR^6-C(O)OR^8, -SR^6, -S(O)R^6, -$S(O)_2R^6$, -$COOR^6$, -C(O)NR^6_2 and -$S(O)_2NR^6_2$.

In a certain aspect of the invention, R is H or selected among the group consisting of a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl, and heteroaryl, said group being substituted with 0-3 R^5 and 0-3 R^9, or selected among the group consisting of C_1-C_3 alkylene-NR^4_2, C_1-C_3 alkylene-NR^4C(O)R^8, C_1-C_3 alkylene-NR^4C(O)OR^8, C_1-C_2 alkylene-O-NR^4_2, C_1-C_2 alkylene-O-NR^4C(O)R^8, and C_1-C_2 alkylene-O-NR^4C(O)OR^8 substituted with 0-3 R^9.

Suitably, R is H or selected among the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl, and heteroaryl, said group being substituted with 0-3 R^5 and 0-3 R^9.

In some aspects of the invention it is preferred that R is selected among the group consisting of C_1-C_3 alkylene-NR^4_2, C_1-C_3 alkylene-NR^4C(O)R^8, C_1-C_3 alkylene-NR^4C(O)OR^8, C_1-C_2 alkylene-O-NR^4_2, C_1-C_2 alkylene-O-NR^4C(O)R^8, and C_1-C_2 alkylene-O-NR^4C(O)OR^8 substituted with 0-3 R^9.

In the present description and claims, the direction of connections between the various components of a building block should be read left to right. For example a spacer is connected to a complementing element through the atom on the left and to the sulphur atom (or alternatively the group A) through the atom on the right hand side.

The term "C_3-C_7 cycloheteroalkyl" as used herein refers to a radical of totally saturated heterocycle like a cyclic hydrocarbon containing one or more heteroatoms selected from nitrogen, oxygen, phosphor, boron and sulphur independently in the cycle such as pyrrolidine (1- pyrrolidine; 2- pyrrolidine; 3- pyrrolidine; 4- pyrrolidine; 5- pyrrolidine); pyrazolidine (1- pyrazolidine; 2- pyrazolidine; 3- pyrazolidine; 4-pyrazolidine; 5-pyrazolidine); imidazolidine (1- imidazolidine; 2- imidazolidine; 3- imidazolidine; 4- imidazolidine; 5- imidazolidine); thiazolidine (2- thia-

zolidine; 3- thiazolidine; 4- thiazolidine; 5- thiazolidine); piperidine (1- piperidine; 2- piperidine; 3- piperidine; 4- piperidine; 5- piperidine; 6- piperidine); piperazine (1- piperazine; 2- piperazine; 3- piperazine; 4- piperazine; 5- piperazine; 6- piperazine); morpholine (2- morpholine; 3- morpholine; 4- morpholine; 5- morpholine; 6- morpholine); thiomorpholine (2- thiomorpholine; 3- thiomorpholine; 4- thiomorpholine; 5- thiomorpholine; 6- thiomorpholine); 1,2-oxathiolane (3-(1,2-oxathiolane); 4-(1,2-oxathiolane); 5-(1,2-oxathiolane); 1,3-dioxolane (2-(1,3-dioxolane); 4-(1,3-dioxolane); 5-(1,3-dioxolane); tetrahydropyrane; (2-tetrahydropyrane; 3-tetrahydropyrane; 4-tetrahydropyrane; 5-tetrahydropyrane; 6-tetrahydropyrane); hexahydropyridazine (1-(hexahydropyridazine); 2-(hexahydropyridazine); 3-(hexahydropyridazine); 4-(hexahydropyridazine); 5-(hexahydropyridazine); 6-(hexahydropyridazine)), [1,3,2]dioxaborolane, [1,3,6,2]dioxazaborocane

The term "aryl" as used herein includes carbocyclic aromatic ring systems of 5-7 carbon atoms. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems as well as up to four fused aromatic- or partially hydrogenated rings, each ring comprising 5-7 carbon atoms.

The term "heteroaryl" as used herein includes heterocyclic unsaturated ring systems containing, in addition to 2-18 carbon atoms, one or more heteroatoms selected from nitrogen, oxygen and sulphur such as furyl, thienyl, pyrrolyl, heteroaryl is also intended to include the partially hydrogenated derivatives of the heterocyclic systems enumerated below.

The terms "aryl" and "heteroaryl" as used herein refers to an aryl which can be optionally substituted or a heteroaryl which can be optionally substituted and includes phenyl, biphenyl, indenyl, naphthyl (1-naphthyl, 2-naphthyl), N-hydroxytetrazolyl, N-hydroxytriazolyl, N-hydroxyimidazolyl, anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), thiophenyl (2-thienyl, 3-thienyl), furyl (2-furyl, 3-furyl), indolyl, oxadiazolyl, isoxazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, thiazolyl, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3- pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-

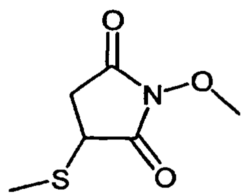
quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl).

The Functional Entity carries elements used to interact with host molecules and optionally reactive elements allowing further elaboration of an encoded molecule of a library. Interaction with host molecules like enzymes, receptors and polymers is typically mediated through van der waal's interactions, polar- and ionic interactions and pi-stacking effects. Substituents mediating said effects may be masked by methods known to an individual skilled in the art (Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 3rd ed.; John Wiley & Sons: New York, 1999.) to avoid undesired interactions or reactions during the preparation of the individual building blocks and during library synthesis. Analogously, reactive elements may be masked by suitably selected protection groups. It is appreciated by one skilled in the

art that by suitable protection, a functional entity may carry a wide range of substituents.

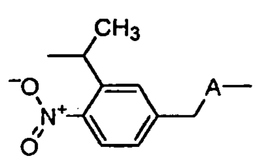
The Functional Entity may be a masked Functional Entity that is incorporated into an encoded molecule. After incorporation, reactive elements of the Functional Entity may be revealed by un-masking allowing further synthetic operations. Finally, elements mediating recognition of host molecules may be un-masked.

The function of the carrier

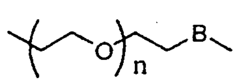


is to provide for the transferability of the functional entity, playing the role of a leaving group.

The spacer serves to distance the functional entity to be transferred from the bulky complementing element. Thus, the identity of the spacer is not crucial for the function of the building block. It may be desired to have a spacer which can be cleaved by light. In this occasion, the spacer is provided with e.g. the group



In the event an increased hydophilicity is desired the spacer may be provided with a polyethylene glycol part of the general formula:



The spacer in conjunction with the carrier makes up a cleavable linker, which links the complementing element to the functional entity.

In a preferred embodiment, the complementing element serves the function of transferring genetic information *e.g.* by recognising a coding element. The recognition implies that the two parts are capable of interacting in order to assemble a complementing element – coding element complex. In the biotechnological field a variety of interacting molecular parts are known which can be used according to the invention. Examples include, but are not restricted to protein-protein interactions, protein-polysaccharide interactions, RNA-protein interactions, DNA-DNA interactions, DNA-RNA interactions, RNA-RNA interactions, biotin-streptavidin interactions, enzyme-ligand interactions, antibody-ligand interaction, protein-ligand interaction, ect.

The interaction between the complementing element and coding element may result in a strong or a week bonding. If a covalent bond is formed between the parties of the affinity pair the binding between the parts can be regarded as strong, whereas the establishment of hydrogen bondings, interactions between hydrophobic domains, and metal chelation in general results in weaker bonding. In general relatively weak bonding is preferred. In a preferred aspect of the invention, the complementing element is capable of reversible interacting with the coding element so as to provide for an attachment or detachment of the parts in accordance with the changing conditions of the media.

In a preferred aspect of the invention, the interaction is based on nucleotides, i.e. the complementing element is a nucleic acid. Preferably, the complementing element is a sequence of nucleotides and the coding element is a sequence of nucleotides capable of hybridising to the complementing element. The sequence of nucleotides carries a series of nucleobases on a backbone. The nucleobases may be any chemical entity able to be specifically recognized by a complementing entity. The nucleobases are usually selected from the natural nucleobases (adenine, guanine, uracil, thymine, and cytosine) but also the other nucleobases obeying the Watson-Crick hydrogen-bonding rules may be used, such as the synthetic nucleobases disclosed in US 6,037,120. Examples of natural and non-natural nucleobases able to perform a specific pairing are shown in Figure 2. The backbone of the sequence of nucleotides may be any backbone able to aggregate the nucleobases is a sequence. Examples of backbones are shown in figure 4. In some aspects of the invention the addition of non-specific nucleobases to the complementing element is advantageous, figure 3.

The coding element can be an oligonucleotide having nucleobases which complements and is specifically recognised by the complementing element, i.e. in the event the complementing element contains cytosine, the coding element part contains guanine and visa versa, and in the event the complementing element contains thymine or uracil the coding element contains adenine.

The complementing element may be a single nucleobase. In the generation of a library, this will allow for the incorporation of four different functional entities into the template-directed molecule. However, to obtain a higher diversity a complementing element preferably comprises at least two and more preferred at least three nucleotides. Theoretically, this will provide for 4^2 and 4^3, respectively, different functional entities uniquely identified by the complementing element. The complementing element will usually not comprise more than 100 nucleotides. It is preferred to have complementing elements with a sequence of 3 to 30 nucleotides.

The building blocks of the present invention can be used in a method for transferring a functional entity to a recipient reactive group, said method comprising the steps of

providing one or more building blocks as described above and

contacting the one or more building blocks with a corresponding coding element associated with a recipient reactive group under conditions which allow for a recognition between the one or more complementing elements and the coding elements, said contacting being performed prior to, simultaneously with, or subsequent to a transfer of the functional entity to the recipient reactive group.

The coding element may comprise one, two, three or more codons, i.e. sequences that may be specifically recognised by a complementing element. Each of the codons may be separated by a suitable spacer group. Preferably, all or at least a majority of the codons of the template are arranged in sequence and each of the codons are separated from a neighbouring codon by a spacer group. Generally, it is preferred to have more than two codons on the template to allow for the synthesis of more complex encoded molecules. In a preferred aspect of the invention the number of codons of the encoding element is 2 to 100. Still more preferred are coding elements comprising 3 to 10 codons. In another aspect, a codon comprises 1 to 50 nucleotides and the complementing element comprises a sequence of nucleotides complementary to one or more of the encoding sequences.

The recipient reactive group may be associated with the encoding element in any appropriate way. Thus, the reactive group may be associated covalently or non-covalently to the coding element. In one embodiment the recipient reactive group is linked covalently to the encoding element through a suitable linker which may be separately cleavable to release the reaction product. In another embodiment, the reactive group is coupled to a complementing element, which is capable of recognising a sequence of nucleotides on the encoding element, whereby the recipient reactive group becomes attached to the encoding element by hybridisation. Also, the recipient reactive group may be part of a chemical scaffold, i.e. a chemical entity having one or more reactive groups available for receiving a functional entity from a building block.

The recipient reactive group may be any group able to cleave the bond between the carrier and the functional entity to release the functional entity. Usually, the reactive group is nucleophilic, such as a hydroxyl, a thiol, an amine etc. A preferred recipient reactive group is an amine group. The nucleophile usually attacks the atom of the functional entity connected to the oxygen attached to the nitrogen ring member of the carrier. When the functional entity is attached to said oxygen through a group X=V, the nucleophile attacks the X atom, thereby causing the carrier group to be a leaving group of the reaction, transferring the X(=V)-Functional entity precursor to the recipient. The chemical structure formed has, in the event the nucleophilic group is an amine attached to a scaffold, the general formula:

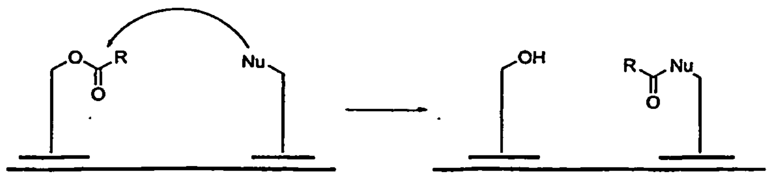
Scaffold-NH-X(=V)-R

In which
X = -C-, -S-, -P-, -S(O)-, -P(O)-, and
V = O, S, NH, N-C_1-C_6 alkyl, and R is as previously defined.

In a preferred aspect X is C and V is O.

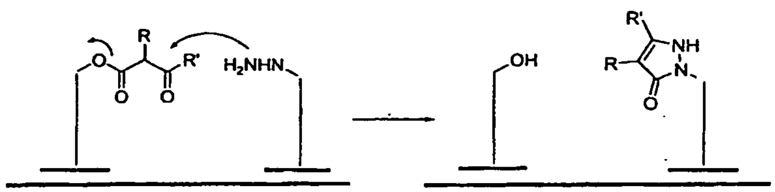
The conditions which allow for transfer to occur are dependent upon the receiving reactive group. Below various examples of the conditions for a transfer to occur are depicted together with the reaction products formed.

A. Acylating building blocks - principle

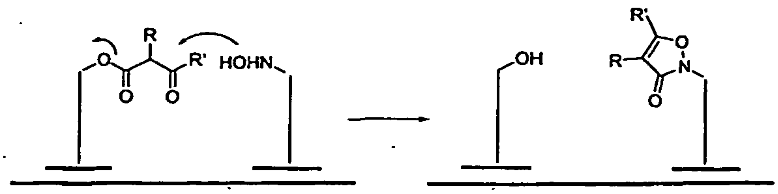


Nu = Oxygen- , Nitrogen- , Sulfur- and Carbon Nucleophiles

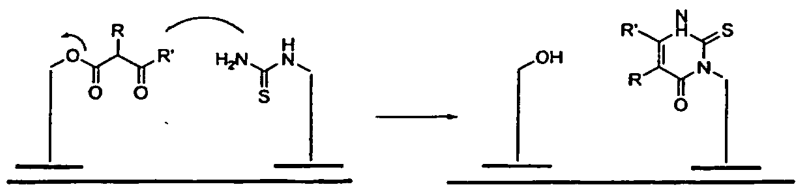
B. Pyrazolone formation by reaction of hydrazines with β–Ketoesters



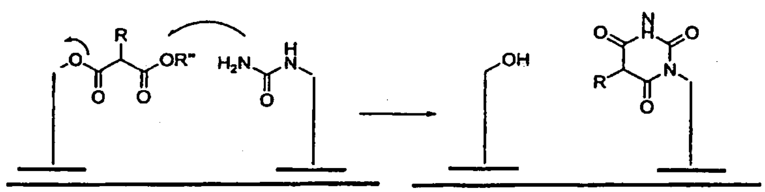
C. Isoxazolone formation by reaction of hydroxylamines with β-Ketoesters



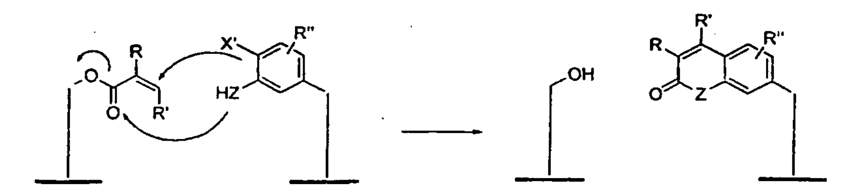
D. Pyrimidine formation by reaction of thioureas with β-Ketoesters



E. Pyrimidine formation by reaction of ureas with Malonates

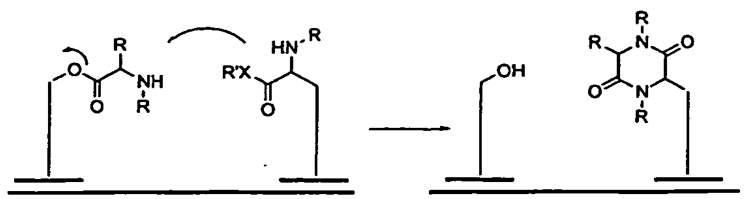


F. Coumarine or quinolinon formation by a Heck reaction followed by a nucleophilic substitution



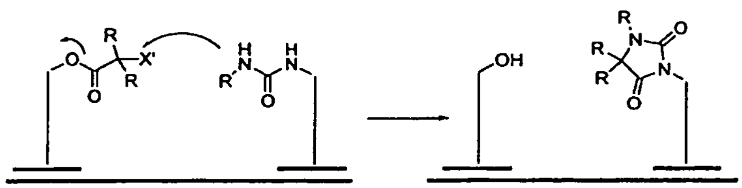
X' = Halogen, OTf, OMs Z = O, NH

G. Diketopiperazine formation by reaction of Amino Acid Esters



R' = H, R

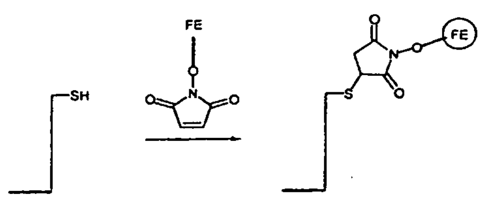
J. Hydantoin formation by reaction of Urea and α-substituted Esters



X' = Hal, OTos, OMs, etc.

The present building blocks may be prepared in accordance with a variety of chemical synthesis schemes. Generally, a complementing element containing a thiol group is provided. In the event, the complementing element is a oligonucleotide, the thiol may be provided during the synthesis of the oligonucleotide by incorporating a suitable nucleotide derivative. When a oligonucleotide comprising a thiol group is desired, a variety of commercial nucleotide derivatives are available, e.g. the C6 S-S thiol modifier (obtainable from Glen Research cat. # 10-1936-90), which may be incorporated using the standard protocol of the phosphoramedite synthesis.

According to a first synthesis scheme the building block can be prepared using the step



The thiol oligonucleotide is reacted with the N-hydroxymaleimide-functional entity derivative via a Michael addition, whereby the SH group is added to the double bond of the maleimide.

According to a second synthesis scheme, the building blocks can be prepared in two step:

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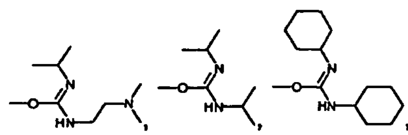
The thiol oligonucleotide is reacted with N-hydroxymaleimide via a Michael addition, whereby the SH group is added to the double bond of the maleimide forming an intermediate oligonucleotide derivative which is reacted further with a functional entity connected to a leaving group (Lg). Preferred leaving groups are


, Cl, Br.

According to a preferred aspect of the invention the building blocks are used for the formation of a library of compounds. The complementing element of the building block is used to identify the functional entity. Due to the enhanced proximity between reactive groups when the complementing entity and the encoding element are contacted, the functional entity together with the identity programmed in the complementing element is transferred to the encoding element associated with recipient reactive group. Thus, it is preferred that the sequence of the complementing element is unique in the sense that the same sequence is not used for another functional entity. The unique identification of the functional entity enable the possibility of decoding the encoding element in order to determine the synthetic history of the molecule formed. In the event two or more functional entities have been transferred to a scaffold, not only the identity of the transferred functional entities can be determined. Also the sequence of reaction and the type of reaction involved can be determined by decoding the encoding element. Thus, according to a preferred embodiment of the invention, each different member of a library comprises a complementing element having a unique sequence of nucleotides, which identifies the functional entity.

Brief description of the drawings

Fig. 1 shows to setups for functional entity transfer.

Fig. 2 shows examples of specific base pairing.

Fig. 3 shows examples of non-specific base-pairing

Fig. 4 shows examples of backbones.

Fig. 5 discloses the results of example 7.

Fig. 6 discloses the results of example 8.

Detailed Description of the Invention

A building block of the present invention is characterized by its ability to transfer its functional entity to a receiving chemical entity. This is done by forming a new covalent bond between the receiving chemical entity and cleaving the bond between the carrier moiety and the functional entity of the building block.

Two setups for generalized functional entity transfer from a building block are depicted in figure 1. In the first example, one complementing element of a building block recognizes a template carrying another functional entity, hence bringing the functional entities in close proximity. This results in a reaction between functional entity 1 and 2 forming a covalent bond between these concurrent with the cleavage of the bond between functional entity 2 and its linker. In the second example, a template brings together two building blocks resulting in functional entity transfer from one building block to the other.

In a library synthesis, several building blocks are mixed in a reaction vessel and the added templates ensure that the building blocks - consequently the functional entities - are combined in the desired manner. As several building blocks are employed at the same time, the use of *in situ* generated building blocks is disfavoured for practical reasons.

Building blocks for library synthesis should posses the necessary reactivity to enable the transfer of the functional entity but should also be stable enough to endure storage and the conditions applied during library synthesis. Hence fine tuning of the reactivity for a particular building block is vital. The reactivity of a building block depends partly on the characteristics of the functional entity and the characteristics of the carrier. E.g. a highly reactive functional entity attached to a highly reactive carrier would form a building block that may be susceptible to hydrolysis during the library synthesis thus preventing successful transfer of one functional entity to another. Further, if transfer of a functional entity precursor is faster than coding element – complementing element recognition unspecific reactions may result.

Therefore, the present invention particularly relates to practically useful library building blocks capable of acting as acylating agents, thioacetylating agents or amidinoylating agents with a balanced reactivity. Such building blocks may be assembled by several different pathways as described below.

The R group of the Functional entity, may be selected from any transferable chemical group capable of forming a connection to –X(=V)- group. In certain aspects of the invention the functional entity precursor is represented by the formula Z^2R^{17}

wherein Z is absent, O, S or NR^{24}. In certain embodiment Z is absent. In a another embodiment Z is O. In still another embodiment Z is S, and in still a further embodiment Z is NR^{24}.

R^{17} and R^{24} independently is H, alkyl, alkenyl, alkynyl, alkadienyl, cycloalkyl, cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of $SnR^{18}R^{19},R^{20}$, $Sn(OR^{18})R^{19}R^{20}$, $Sn(OR^{18})(OR^{19})R^{20}$, $BR^{18}R^{19}$, $B(OR^{18})R^{19}$, $B(OR^{18})(OR^{19})$, halogen, CN, CNO, $C(halogen)_3$, OR^{18}, $OC(=O)R^{18}$, $OC(=O)OR^{18}$, $OC(=O)NR^{18}R^{19}$, SR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, N_3, $NR^{18}R^{19}$, $N^+R^{18}R^{19}R^{20}$, $NR^{18}OR^{19}$, $NR^{18}NR^{19}R^{20}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, NC, $P(=O)(OR^{18})OR^{19}$, $P^+R^{18}R^{19}R^{20}$, $C(=O)R^{18}$, $C(=NR^{18})R^{19}$, $C(=NOR^{18})R^{19}$, $C(=NNR^{18}R^{19})$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$, $C(=O)NR^{18}NR^{19}R^{20}$, $C(=NR^{18})NR^{19}R^{20}$, $C(=NOR^{18})NR^{19}R^{20}$ or R^{21},

wherein,

R^{18}, R^{19} and R^{20} independently is H, alkyl, alkenyl, alkynyl, alkadienyl, cycloalkyl, cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of halogen, CN, CNO, $C(halogen)_3$, OR^{21}, $OC(=O)R^{21}$, $OC(=O)OR^{21}$, $OC(=O)NR^{21}R^{22}$, SR^{21}, $S(=O)R^{21}$, $S(=O)_2R^{21}$, $S(=O)_2NR^{21}R^{22}$, NO_2, N_3, $NR^{21}R^{22}$, $N^+R^{21}R^{22}R^{23}$, $NR^{18}OR^{19}$, $NR^{18}NR^{19}R^{20}$, $NR^{21}C(=O)R^{22}$, $NR^{21}C(=O)OR^{22}$, $NR^{21}C(=O)NR^{22}R^{23}$, NC, $P(=O)(OR^{21})OR^{22}$, $P^+R^{18}R^{19}R^{20}$, $C(=O)R^{21}$, $C(=NR^{21})R^{22}$, $C(=NOR^{21})R^{22}$, $C(=NNR^{21}R^{22})$, $C(=O)OR^{21}$, $C(=O)NR^{21}R^{22}$, $C(=O)NR^{21}OR^{22}$ $C(=NR^{18})NR^{19}R^{20}$, $C(=NOR^{18})NR^{19}R^{20}$or $C(=O)NR^{21}NR^{22}R^{23}$, wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

wherein,

R^{21}, R^{22} and R^{23} independently is H, alkyl, alkenyl, alkynyl, alkadienyl, cycloalkyl, cycloheteroalkyl, aryl or heteroaryl and wherein R^{21} and R^{22} may together form a 3-8 membered heterocyclic ring or R^{21} and R^{23} may together form a 3-8 membered heterocyclic ring or R^{22} and R^{23} may together form a 3-8 membered heterocyclic ring,

In a further embodiment,

R^{17} and R^{24} independently is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of $SnR^{18}R^{19},R^{20}$, $Sn(OR^{18})R^{19}R^{20}$, $Sn(OR^{18})(OR^{19})R^{20}$, $BR^{18}R^{19}$, $B(OR^{18})R^{19}$, $B(OR^{18})(OR^{19})$, halogen, CN, CNO, $C(halogen)_3$, OR^{18}, $OC(=O)R^{18}$, $OC(=O)OR^{18}$, $OC(=O)NR^{18}R^{19}$, SR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, N_3, $NR^{18}R^{19}$, $N^+R^{18}R^{19}R^{20}$, $NR^{18}OR^{19}$, $NR^{18}NR^{19}R^{20}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, NC, $P(=O)(OR^{18})OR^{19}$, $P^+R^{18}R^{19}R^{20}$, $C(=O)R^{18}$, $C(=NR^{18})R^{19}$, $C(=NOR^{18})R^{19}$, $C(=NNR^{18}R^{19})$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$, $C(=O)NR^{18}NR^{19}R^{20}$, $C(=NR^{18})NR^{19}R^{20}$, $C(=NOR^{18})NR^{19}R^{20}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_8 alkadienyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In another embodiment,

R^{17} and R^{24} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of halogen, CN, $C(halogen)_3$, OR^{18}, $OC(=O)R^{18}$, $OC(=O)OR^{18}$, $OC(=O)NR^{18}R^{19}$, SR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}OR^{19}$, $NR^{18}NR^{19}R^{20}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $P(=O)(OR^{18})OR^{19}$, $C(=O)R^{18}$, $C(=NR^{18})R^{19}$, $C(=NOR^{18})R^{19}$, $C(=NNR^{18}R^{19})$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$, $C(=O)NR^{18}NR^{19}R^{20}$, $C(=NR^{18})NR^{19}R^{20}$, $C(=NOR^{18})NR^{19}R^{20}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $OC(=O)R^{18}$, $OC(=O)OR^{18}$, $OC(=O)NR^{18}R^{19}$, SR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}OR^{19}$, $NR^{18}NR^{19}R^{20}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $P(=O)(OR^{18})OR^{19}$, $C(=O)R^{18}$, $C(=NR^{18})R^{19}$, $C(=NOR^{18})R^{19}$, $C(=NNR^{18}R^{19})$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$, $C(=O)NR^{18}NR^{19}R^{20}$, $C(=NR^{18})NR^{19}R^{20}$, $C(=NOR^{18})NR^{19}R^{20}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl,

phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or morpholinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, phenyl or naphtyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or morpholinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, phenyl or naphtyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl optionally substituted with one or more substituents se-

lected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl or butyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or morpholinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl or butyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,
R^{17} and R^{24} independently is H, phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},
wherein,
R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl or butyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, phenyl or naphtyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl or butyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is H, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, methyl, ethyl, propyl or butyl and wherein R^{18} and R^{19} may together form a 3-8 membered heterocyclic ring or R^{18} and R^{20} may together form a 3-8 membered heterocyclic ring or R^{19} and R^{20} may together form a 3-8 membered heterocyclic ring,

In still another embodiment,

R^{17} and R^{24} independently is methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl,

In still another embodiment,

R^{17} and R^{24} independently is aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or morpholinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl,

In still another embodiment,

R^{17} and R^{24} independently is phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl,

In still another embodiment,

R^{17} and R^{24} independently is phenyl or naphtyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl,

In still another embodiment,

R^{17} and R^{24} independently is thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl,

In still another embodiment,

R^{17} and R^{24} independently is methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl,

In still another embodiment,

R^{17} and R^{24} independently is aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or morpholinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl,

In still another embodiment,

R^{17} and R^{24} independently is phenyl, naphtyl, thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl,

In still another embodiment,

R^{17} and R^{24} independently is phenyl or naphtyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},.

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl,

In still another embodiment,

R^{17} and R^{24} independently is thienyl, furyl, pyridyl, quinolinyl or isoquinolinyl optionally substituted with one or more substituents selected from the group consisting of F, Cl, CN, CF_3, OR^{18}, $S(=O)R^{18}$, $S(=O)_2R^{18}$, $S(=O)_2NR^{18}R^{19}$, NO_2, $NR^{18}R^{19}$, $NR^{18}C(=O)R^{19}$, $NR^{18}C(=O)OR^{19}$, $NR^{18}C(=O)NR^{19}R^{20}$, $C(=O)R^{18}$, $C(=NOR^{18})R^{19}$, $C(=O)OR^{18}$, $C(=O)NR^{18}R^{19}$, $C(=O)NR^{18}OR^{19}$ or R^{21},

wherein,

R^{18}, R^{19}, R^{20} and R^{21} independently is H, phenyl, naphthyl, thienyl, furyl, pyridinyl, quinolinyl or isoquinolinyl,

In still another embodiment,

R^{17} and R^{24} independently is H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 cycloheteroalkyl, aryl or heteroaryl

In still another embodiment,

R^{17} and R^{24} independently is H,

In still another embodiment,

R^{17} and R^{24} independently is C_1-C_6 alkyl, C_3-C_7 cycloalkyl or C_3-C_7 cycloheteroalkyl,

In still another embodiment,

R^{17} and R^{24} independently is methyl, ethyl, propyl or butyl

in still another prefered embodiment

R^{17} and R^{24} independently is cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl

in still another prefered embodiment
R^{17} and R^{24} independently is aziridinyl, pyrrolidinyl, piperidinyl or morpholinyl

In still another embodiment,
R^{17} and R^{24} independently is aryl or heteroaryl

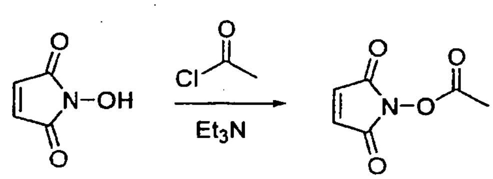
In still another embodiment,
R^{17} and R^{24} independently is phenyl or naphthyl

In still another embodiment,
R^{17} and R^{24} independently is thienyl, furyl, pyridyl, quinolinyl or isoquinolyl

Experiments

All oligos used were prepared by standard phosphoramidite chemistry and purchased from DNA technology, Denmark. The type II compounds used were commercially available from Fluka (4-pentynoic acid cat. no: 77055, 5-hexynoic acid cat. no: 53108 and *N*-tertbutoxycarbonyl beta-alanin cat. no: 15382). The hexapeptide used as scaffold was synthesised using standard Fmoc chemistry and protected at the N-terminal by acetylation and at the C-terminal by formamide formation. The protected hexapeptide was commercially available from Schaefer-*N*, Denmark.

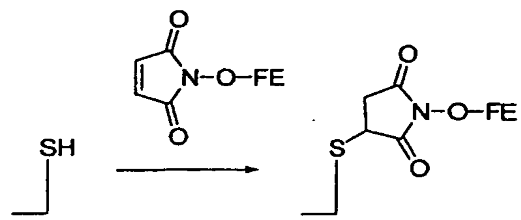
Example 1: Preparation of type I compound (method A)



N-hydroxymaleimide (4 mmol) was mixed with Et_3N (4 mmol) in DCM (15 mL) at 0 °C. Acetyl chloride (4 mmol) was added and the reaction mixture was left at rt o/n. DCM (15 mL) was added and the reaction mixture was washed with citric acid (3 x 30 mL), $NaHCO_3$ (2 x 30 mL) and NaCl aq. (30 mL). The organic phase was dried

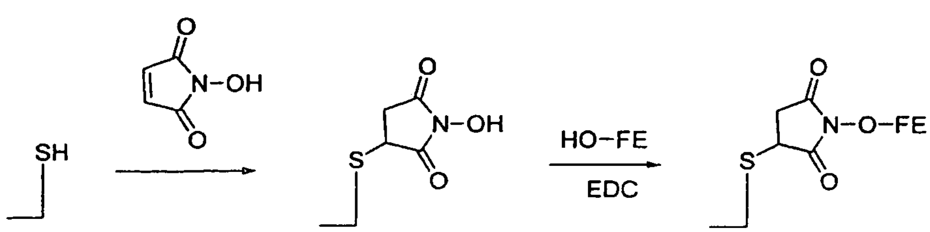
over $MgSO_4$ and evaporated *in vacuo* to afford acetic acid 2,5-dioxo-2,5-dihydropyrrol-1-yl ester in 41% yield. ^1H NMR ($CDCl_3$): 6.74 (s, 2H), 2.32 (s, 2H).

Example 2: Preparation of building blocks (method A)



A dTS-S-oligo (10 nmol) is evaporated to dryness *in vacuo*. The oligo is redissolved in DTT (50 µl 100 mM) in 100 mM Sodium-phosphate buffer pH 8.0. Incubate at 37 °C for 1h and purify using a micro-spin column equilibrated with Hepes-OH (100 mM, pH 7.5). The HS-oligo is treated with CTAB (50 µL, 1 mM) and the mixture is evaporated to dryness *in vacuo*. The HS-oligo obtained is redissolved in DMF (100 µL) and treated with compounds of type I (100 µl 100 mM in DMF) for 3h at rt. NaOAc (200 µl 1 M, pH = 7.5) is added and the reaction mixture is extracted with EtOAc (2 x 300 µL). The loaded oligo is finally purified using a micro-spin column equilibrated with Hepes-OH (100 mM, pH 7.5).

Example 3: Preparation of building blocks (method B)



C_6S-S-oligonucleotides A to D (10 nmol) is evaporated to dryness *in vacuo*.

A: 5'-GCG ACC TGG AGC ATC CAT CGT **S**
B: 5'-GAG CAT CCA TCG **S**
C: 5'-GAC GAG CAT CCA TCG **S**
D: 5'-CTA GGG ACG AGC ATC CAT CG**S**

S = Thiol C6 SS modifier (Glen# 10-1936)

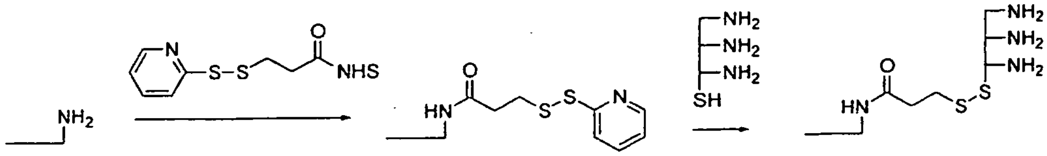
The oligo is redissolved in DTT (50 µl 100 mM) in 100 mM Sodium-phosphate pH 8.0. Incubate at 37 °C for 1h and purify using a micro-spin column equilibrated with Hepes-OH (100 mM, pH 7.5). NHM (50 µl 100 mM) in Hepes-OH (100mM, pH 7.5) is added to the obtained HS-oligo and the mixture is incubated at 25°C for 2h. The oligo-S-NHS is then purified using a Microspin columns equilibrated in MS-grade H_2O and analysed by ES-MS.

A: MS (calc): 6723.52; MS (found): 6723.21
B: MS (calc): 3938.75; MS (found): 3937.78
C: MS (calc): 4870.36; MS (found): 4869.42
D: MS (calc): 6435.38; MS (found): 6434.57

Four EDC-activated compounds were prepared by mixing 50 µL 100mM of each of the compounds (acetic acid, 4-pentynoic acid, N-tertbutoxycarbonyl beta-alanine, and 5-hexynoic acid) in DMF with 50 µl 100 mM of EDC in DMF and leave the mixture at rt for 30 min before use. Subsequently, each of the oligo-S-NHS (1 nmol) is redissolved in MES-buffer (10 µl 100 mM, pH 6) and treated with 10 µl of a DMF solution of the EDC-activated compounds. After 1 h the building blocks are purified using a microspin column equilibrated with 100 mM MES pH6 to obtain
oligonucleotide A loaded with acetyl,
oligonucleotide B loaded with 4-pentynyl (=FE_1),
oligonucleotide C loaded with N-tertbutoxycarbonyl beta-alaninyl (=FE_2), and
oligonucleotide D loaded with 5-hexynyl (FE_3).

ES-MS analysis of the loaded oligonucleotides showed the masses of their corresponding oligo-S-NHS-building blocks shown above, due to the presence of piperidine added during analysis.

Example 4: Preparation of scaffold building blocks



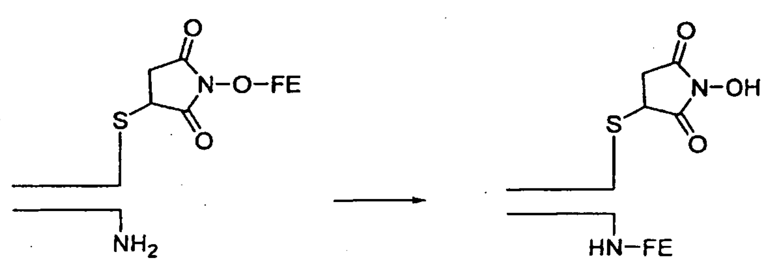
10 nmol of the amino-oligo was diluted in 160 µL 100 mM Hepes-KOH buffer pH 7.5. *N*-Succinimidyl 3-[2-pyridyldithio]-propionamido, SPDP (40 µl 20 mM, Pierce cat # 21857) was added and the mixture was incubated for 2 h at 30°C. The oligo was extracted with ethyl acetate (200 µL) and purified using micro spin columns equilibrated with 100 mM Hepes-KOH buffer pH 7,5. The hexapeptide CysPhePheLys-LysLys (10 µl 100 mM) was added and the mixture was incubated over-night at 30°C. The oligo was purified by ammoniumacetate precipitation and analysed by ES-MS.

MS (calc): 8386.41; MS (found): 8386.57

Used oligo:
E: 5'-**X** CGA TGG ATG CTC GTC CCT AGA **YZ**

X = 5'-amino modifier C6 (Glen# 10-1926)
Y = PC spacer (Glen# 10-4913)
Z = Biotin phosphoramidite (Glen# 10-1955)

Example 5: Transfer of a Functional entity



Oligonucleotide A loaded with acetyl (250 pmol) was added to oligo F (200 pmol) in 50 µl 100 mM MES, pH 6. The mixture was incubated overnight at 25 °C. Subsequently, the mixture was purified by gel filtration using a microspin column equilibrated with H_2O and transfer of the functional entity was verified by electron spray mass spectrometry (ES-MS).

Used oligos:

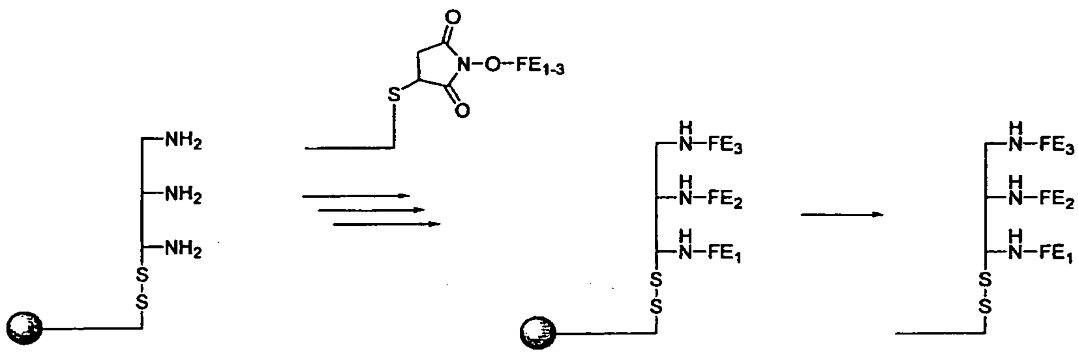
A: 5'-GCG ACC TGG AGC ATC CAT CGT - acetyl

F: 5'- X ACG ATG GAT GCT CCA GGT CGC

X = 5' Amino-modifier C6 (Glen# 10-1906)

MS (calc): 6667.46; MS (found) 6666.64.

Example 6: Transfer of a three different Functional entities



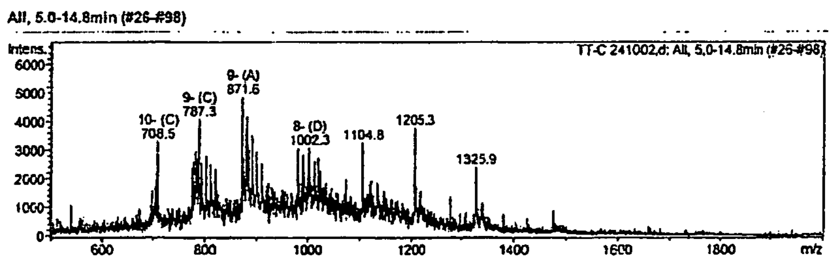
Transfer of the first functional entity: Scaffold building block oligo E (400 pmol) was added to oligo B (400 pmol in 25 µl MES buffer, pH 6), loaded with 4-pentynyl, and incubated over-night at 15°C. The volume was then adjusted to 50 µl and the mixture transferred to a streptavidin-bead slurry (Pharmacia cat #17-5113-01, pre-washed with 100 ul MES buffer) and incubated for 10 min at room-temperature, followed by incubation on ice for 10 min. The beads were washed four times with ddH_2O, resuspended in 100 µl 10mM NaOH and incubated for 2 min at room temperature to denature the duplex. The NaOH was removed and the beads were subsequently washed twice with 60°C ddH_2O. The water was removed and the beads resuspended in 25 µl 100 mM MES buffer pH 6.0.

Transfer of the second functional entity: Oligo C (400 pmol in 25 µl MES buffer, pH 6), loaded with *N*-tertbutoxycarbonyl beta-alaninyl, was added to the beads and the mixture was incubated at 25°C for 2h. The beads were washed four times with ddH_2O, resuspended in 100 µl 10mM NaOH and incubated for 2 min at room temperature to denature the duplex. The NaOH was removed and the beads were subsequently washed twice with 60°C ddH_2O. The water was removed and the beads resuspended in 25 µl 100 mM MES buffer pH 6.0.

Transfer of the third functional entity: Oligo D (400 pmol in 25 µl MES buffer, pH 6), loaded with 5-hexynyl, was added to the beads and the mixture was incubated at 25°C for 2h. The beads were washed four times with ddH_2O, resuspended in 100 µl 10mM NaOH and incubated for 2 min at room temperature to denature the duplex. The NaOH was removed and the beads were subsequently washed twice with 60°C ddH_2O. The beads were additionally washed once with 50 µl MES buffer and twice with 50 µL water. The beads were resuspended in 25 µl ddH_2O and put on UV transilluminator for 2x15 seconds to cleave oligo E from the beads. 25 µl 12% ammonia was added and the mixture was incubated for 5 min at 50°C. The sample was spun twice at 5kG, and the supernatant collected. The sample was evaporated to dryness *in vacuo*, and analysed by ES-MS.

MS of the trisubstituted product (calc): 8197.17

MS of the trisubstituted product (found): 8196.80



	Component	Deconvoluted Mass	Molecule	Absolute Abundance	Relative Abundance
Oligo E	A	7852.08	[M - H]-	10521	80.80
1st round product	B	7931.87	[M - H]-	9513	73.05
	C	7093.34	[M - H]-	12164	93.41
1+3 round product	D	8025.63	[M - H]-	9285	71.30
	E	7037.95	[M - H]-	7630	58.59
	F	8830.74	[M - H]-	8214	63.08
1+2 round product	G	8104.15	[M - H]-	7649	58.74
	H	7094.60	[M - H]-	7213	55.39
Trisubstituted product	I	8196.80	[M - H]-	6771	52.00

Example 7: Attachment of functional entity to a thio oligo.

The following oligos containing a nucleobase modified with a *S*-triphenylmethyl protected thio moiety, were synthesised using the conventional phosphoramidite approach:

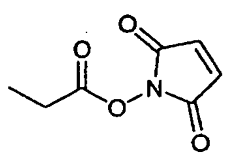
L: 5'-WCA TTG ACC TGA ACC ATG BTA AGC TGC CTG TCA GTC GGT ACT ACG ACT ACG TTC AGG CAA GA

M: 5'-WCA TTG ACC TGA ACC ATG TBA AGC TGC CTG TCA GTC GGT ACT TCA AGG ATC CAC GTG ACC AG

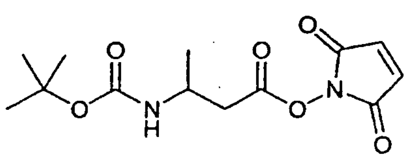
W was incorporated using the commercially available thiol modifier phosphoramidite (10-1926-90 from Glen research). B is an internal biotin incorporated using the commercially available phosphoramidite (10-1953-95 from Glen research).

To make an SH group available for further reaction, the *S*-triphenylmethyl protected thio oligo (10 nmol) was evaporated *in vacuo* and resuspended in TEAA buffer (200 uL of a 0.1M solution, pH=6.4). $AgNO_3$ (30 uL of a 1 M solution) was added and the mixture was left at room temperature for 1-2 hours. DTT (46 uL of a 1M solution) was added and left for 5-10 minutes. The reaction mixture was spun down (20.000 G for 20 minutes) and the supernatant was collected. The solid was extracted with additional TEAA buffer (100 ul of a 0.1 M solution, pH=6.4). The pure thio oligo was obtained by conventional EtOH-precipitation.

The L oligo was subsequently reacted with the compound

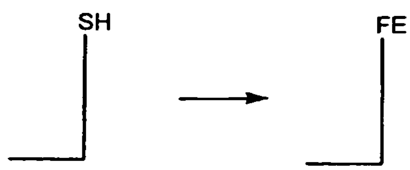


forming a building block able to transfer an acetyl group to a nucleophilic group like an amine, and the M oligo was reacted with the compound



forming a building block capable of transferring a 3-tertbutoxycarbonylamino-butanyl group to a nucleophilic recipient group.

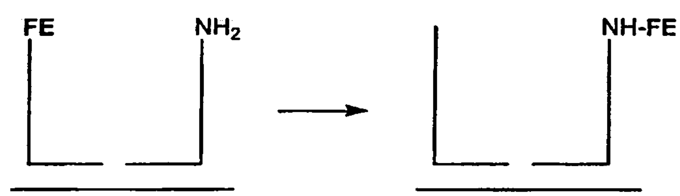
The reaction may be represented by the reaction scheme:



General procedure: The thio oligo (1 nmol) was dried *in vacuo* and treated with the NHS compound shown above in dimethylformamide (50 ul of a 0.1 M solution) and left o/n at rt. The thio oligo was spun down (20.000 G for 10 minutes) and the supernatant removed. Dimethylformamide (1 mL) was added and the loaded thio oligo was spun down (20.000 G for 10 minutes). The dimethylformamide was removed and the loaded thio oligo was resuspended in TEAA buffer (25 uL of a 0.1M solution, pH=6.4) and analysed by HPLC.

The functional entities were transferred to a amino oligonucleotide according to the scheme:

Template



General procedure: The template oligo 5'-BTCTTGCCTGAACGTAGTCGTAGGTCGATCCGCGTTACCAGAGCTGGATGCTCGACAGGTCCCGATGCAATCCAGAGGTCG (1 nmol) was mixed with the oligos (L or M) loaded with a functional entity (1 nmol) and amino oligo O in hepes-buffer (20 uL of a 100 mM HEPES and 1 M NaCl solution, pH=7.5) and water (added to a final volume of 100 uL). The oligos were annealed to the template by heating to 50 °C and cooled (-2 °C/ 30 second) to 30 °C. The mixture was then left o/n at a fluctuating temperature (10 °C for 1 second then 35 °C for 1 second). The oligo complex was attached to streptavidine by addition of streptavidine beads (100 uL, prewashed with 2x1 mL 100 mM hepes buffer and 1M NaCl , pH=7.5). The beads were washed with hepes buffer (1mL). The amino oligo was separated from the streptavidine bound complex by addition of water (200 uL) followed by heating to 70 °C for 1

minute. The water was transferred and evaporated *in vacuo*, resuspended in TEAA buffer (45 uL of a 0.1 M solution) and product formation analysed by HPLC (see Figure 5).

Figure 5 shows the transfer of functional entities to an oligo containing a modified nucleobase with an amino group.
A) The top chromatogram show the reference amino oligo O: 5'-GAC CTG TCG AGC ATC CAG CTT CAT GGC TGA GTC CAC AAT GZ. Z contain the modified nucleobase with an aminogroup, incorporated using the commercially available amino modifier C6 dT phosphoramidite (10-1039-90 from Glen research).
B) The middle chromatogram show the streptavidine purified amino oligo O after partial transfer of a acetyl group from oligo L.
C) The bottom chromatogram show the streptavidine purified amino oligo O after the complete transfer of the more lipophilic 3-tertbutoxycarbonylamino-butanyl.
The following gradient was used in the obtainment of the chromatograms: 0-3 minutes 100% A then 15% A and 85% B from 3-10 minutes.

The experiment where the template oligo was omitted showed no non-templated product formation. The results indicate that the efficiency of the templated synthesis was 80-100%. The reason for less than 100% efficiency was probably due to hydrolytic cleavage of the functional entity.

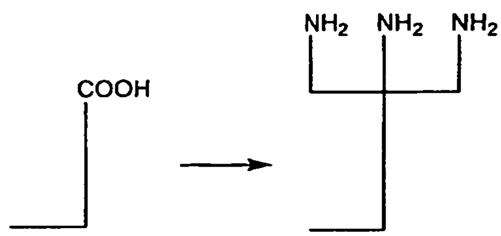
Example 8: Simultaneous transfer of two functional entities

The following oligo containing a nucleobase modified with a carboxylic acid moiety, was synthesised using the conventional phosphoramidite approach:

H: 5'-GAC CTG TCG AGC ATC CAG CTT CAT GGG AAT TCC TCG TCC A<u>CA ATG</u> XT

X was incorporated using the commercially available carboxy-dT phosphoramidite (10-1035-90 from Glen research).

The modified oligo was provided with a trisamine scaffold according to the scheme:



Procedure: The modified oligo (1 nmol) was mixed with water (100 uL), hepes buffer (40 uL of a 200 mM, pH=7.5), NHS (20 uL of a 100 mM solution), EDC (20 uL of a freshly prepared 1 M solution) and the tetraamine tetrakis(aminomethyl)methane tetrahydrochloride (20 uL of a 100 mM solution). The reaction mixture was left o/n at room temperature. The volume was reduced to 60 uL by evaporation *in vacuo*. The pure oligo was obtained by addition of NH_3 conc. (20 uL) followed by HPLC purification. It was possible to isolate a peak after approximately 6 min using the following gradient: : 0-3 minutes 100% A then 15% A and 85% B from 3-10 minutes then 100% B from 10-15 minutes then 100% A from 15-20 minutes. A = 2% acetonitrile in 10 mM TEAA and B = 80% acetonitrile in 10 mM TEAA.

The following oligos containing a nucleobase modified with a *S*-triphenylmethyl protected thio moiety, was synthesised using the conventional phosphoramidite approach:

K: 5'-WCA TTG ACC TGT CTG CCB TGT CAG TCG GTA CTG TGG TAA CGC GGA TCG ACC T

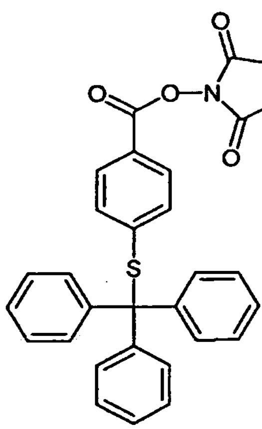
L: 5'-WCA TTG ACC TGA ACC ATG BTA AGC TGC CTG TCA GTC GGT ACT ACG ACT ACG TTC AGG CAA GA

W was incorporated using the commercially available thiol modifier phosphoramidite (10-1926-90 from Glen research). B is an internal biotin incorporated using the commercially available phosphoramidite (10-1953-95 from Glen research).

To make an SH group available for further reaction, the *S*-triphenylmethyl protected thio oligo (10 nmol) was evaporated *in vacuo* and resuspended in TEAA buffer (200

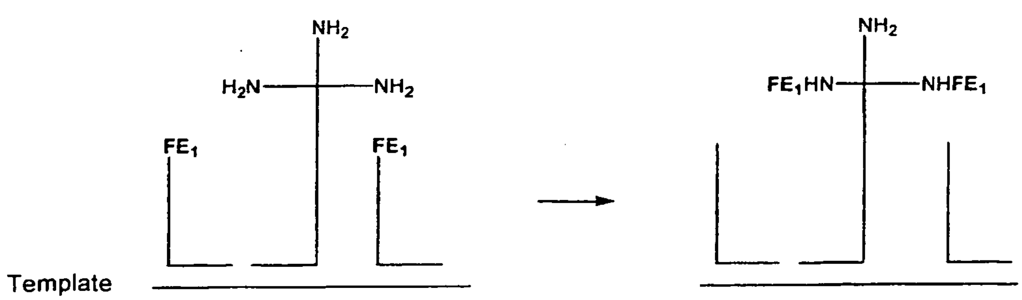
uL of a 0.1M solution, pH=6.4). $AgNO_3$ (30 uL of a 1 M solution) was added and the mixture was left at room temperature for 1-2 hours. DTT (46 uL of a 1M solution) was added and left for 5-10 minutes. The reaction mixture was spun down (20.000 G for 20 minutes) and the supernatant was collected. The solid was extracted with additional TEAA buffer (100 ul of a 0.1 M solution, pH=6.4). The pure thio oligo was obtained by conventional EtOH-precipitation.

The K and L oligo was subsequently reacted with the compound



forming a building block capable of transferring the lipophilic S-Trityl-4-mercaptobenzoyl group to a recipient nucleophilic group.

The transfer reaction is schematically represented below:



The template oligo 5'-BTCTTGCCTGAACGTAGTCGTAGGTCGATCCGCGTTACCAGAGCTGGATGCTC

GACAGGTCCCGATGCAATCCAGAGGTCG (1 nmol) was mixed with the two thio oligos (K and L) loaded with the same functional entity (S-Trityl-4-mercaptobenzoyl; 1 nmol) and the trisamine oligo H (1 nmol) in hepes-buffer (20 uL of a 100 mM hepes and 1 M NaCl solution, pH=7.5) and water (added to a final volume of 100 uL). The oligos were annealed to the template by heating to 50 °C and cooled (-2 °C/ 30 second) to 30 °C. The mixture was then left o/n at a fluctuating temperature (10 °C for 1 second then 35 °C for 1 second). The oligo complex was attached to streptavidine by addition of streptavidine beads (100 uL, prewashed with 2x1 mL 100 mM hepes buffer and 1M NaCl , pH=7.5). The beads were washed with hepes buffer (1mL). The trisamine scaffold oligo H was separated from the streptavidine bound complex by addition of water (200 uL) followed by heating to 70 °C. The water was transferred and evaporated *in vacuo*, resuspended in TEAA buffer (45 uL of a 0.1 M solution) and product formation analysed by HPLC (see Figure 6).

The HPLC chromatogram shows the transfer of two functional entities to a scaffold oligo with three amino groups.

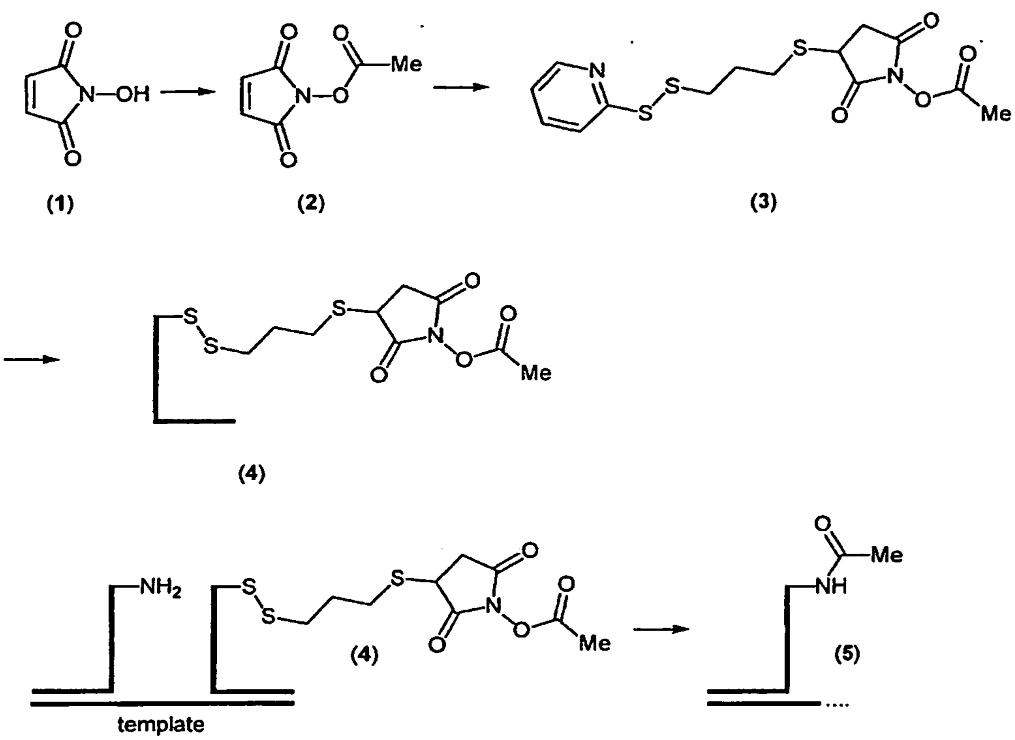
A) The top chromatogram shows the reference scaffold oligo H.

B) The bottom chromatogram show the streptavidine purified scaffold oligo H after the partial transfer of one (peak at 7.94 minutes) and two (peak at 10.76 minutes) identical functional entities (S-Trityl-4-mercaptobenzoyl). The following gradient was used: 0-3 minutes 100% A, then 15% A, and 85% B from 3-10 minutes then 100% B from 10-15 minutes. A = 2% acetonitrile in 10 mM TEAA and B = 80% acetonitrile in 10 mM TEAA.

Due to the lipophilic nature of the functional entities a longer retention time, in the HPLC chromatogram of the scaffolded molecule with two functional entities compared to one functional entity, was observed. The efficiency of the templated synthesis of a scaffolded molecule with the two identical functional entities was about 25% (peak at 10.76 minutes in Figure 6).

Model Example 1

General route to the formation of acylating building blocks and the use of these:



N-hydroxymaleimide (**1**) may be acylated by the use of an acylchloride e.g. acetylchloride or alternatively acylated in e.g. THF by the use of dicyclohexylcarbodiimide or diisopropylcarbodiimide and acid e.g. acetic acid. The intermediate may be subjected to Michael addition by the use of excess 1,3-propanedithiol, followed by reaction with either 4,4'-dipyridyl disulfide or 2,2'-dipyridyl disulfide. This intermediate (**3**) may then be loaded onto an oligonucleotide carrying a thiol handle to generate the building block (**4**). The reaction of this building block with an amine carrying scaffold is conducted as follows:

The template oligonucleotide (1 nmol) is mixed with a thio oligonucleotide building block e.g. (**4**) (1 nmol) and an amino-oligonucleotide scaffold (1 nmol) in hepes-buffer (20 μL of a 100 mM hepes and 1 M NaCl solution, pH=7.5) and water (39 uL). The oligonucleotides are annealed to the template by heating to 50 °C and cooling

(2 °C/ second) to 30 °C. The mixture is then left o/n at a fluctuating temperature (10 °C for 1 second then 35 °C for 1 second), to yield template bound (5).

The above examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full content of this document, including the examples shown above and the references to the scientific a patent literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The examples above contain important additional information that can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

Abbreviations

DCC	N,N'-Dicyclohexylcarbodiimide
DhbtOH	3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
DIC	Diisopropylcarbodiimide
DIEA	Diethylisopropylamin
DMAP	4-Dimethylaminopyridine
DNA	Deoxyribosenucleic Acid
EDC	1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide·HCl
HATU	2-(1*H*-7-Azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOAt	N-Hydroxy-7-azabenzotriazole
HOBt	N-Hydroxybenzotriazole
LNA	Locked Nucleic Acid
NHS	N-hydroxysuccinimid
OTf	Trifluoromethylsulfonate
OTs	Toluenesulfonate
PNA	Peptide Nucleic Acid
PyBoP	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoro-phosphate
PyBroP	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
RNA	Ribonucleic acid
TBTU	2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetra-fluoroborate
TEA	Triethylamine
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
TBDMS-Cl	*Tert*-Butyldimethylsilylchloride
5-Iodo-dU	5-iodo-deoxyriboseuracil
TLC	Thin layer chromatography
$(Boc)_2O$	Boc anhydride, di-*tert*-butyl dicarbonate
TBAF	Tetrabutylammonium fluoride
SPDP	Succinimidyl-propyl-2-dithiopyridyl
CTAB	Cetylammoniumbromide